Bioluminescence-based approach to monitor neural activity in freely moving Drosophilalarvae

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Bioluminescence-based approach to monitor neural activity in freely moving *Drosophila* larvae

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Background:

Dissecting behvaioural circuits requires us to examine activity in the brain as the animal processes sensory information and generates autonomous behaviour. With a few exceptions most current methods require a constrained preparation, which does not translate well to application in freely moving animals. We developed a non-invasive bioluminescence-based approach to address this problem; targeting the expression of the calcium-reporter Aequorin (AEQ) to the *Drosophila* larval nervous system to measure neural activity. We report conditions that significantly increase the sensitivity of this assay, allowing us to measure activity in Kenyon cells (100s) as well as in smaller populations of neurons, with clear signals obtained from less that 10 neurons in the living, unconstrained animal. Finally, we measure both spontaneous activity and evoked responses as the animal encounters specific environmental stimuli (e.g. tastes, odours).

Methods: Bioluminescence principle

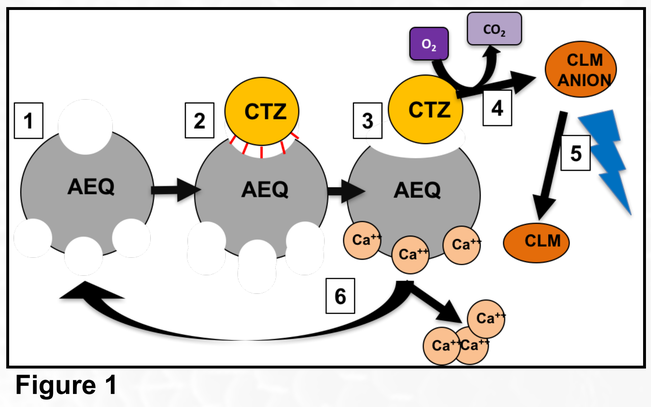

Figure 1

Figure 1. The principle of bioluminescence in *Drosophila* larvae in six steps. AEQ= Aequorin, CTZ= Coelenterazine, CLM= Coeletnteramide. In red the covalent bound between CTZ and AEQ, that are broken when Ca^{++} bind AEQ.

Results (I): Detection of neural activity in intact larvae

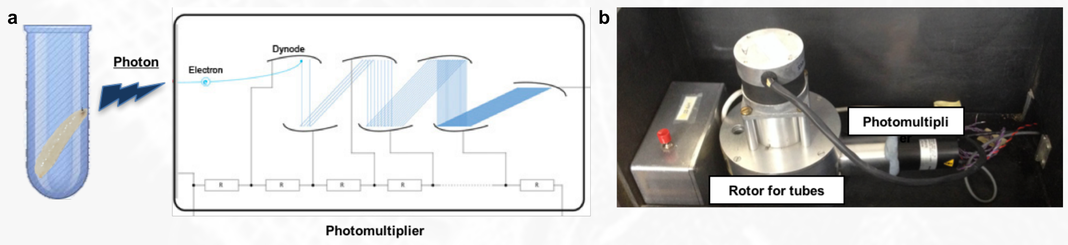

Figure 2. The set-up used to record bioluminescence from neurons in intact *Drosophila* larvae.

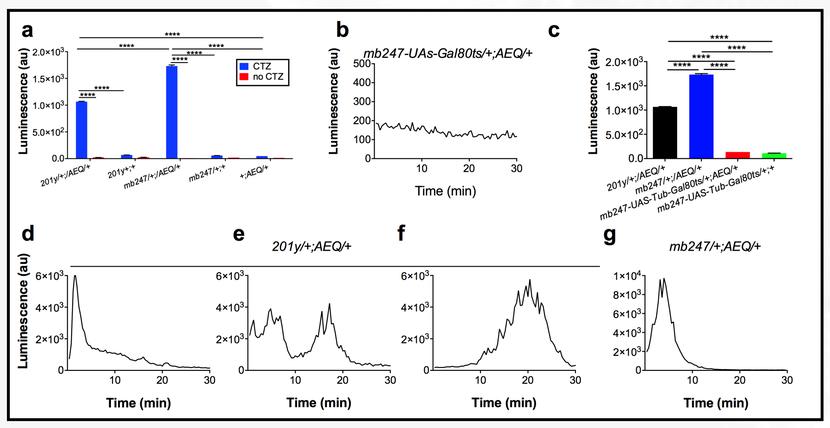

Figure 3. Detection of spontaneous activity of Kenyon cells from intact larvae. **a** and **c** represent the mean bioluminescence values, while **c, d-g** are representative indvidual examples.

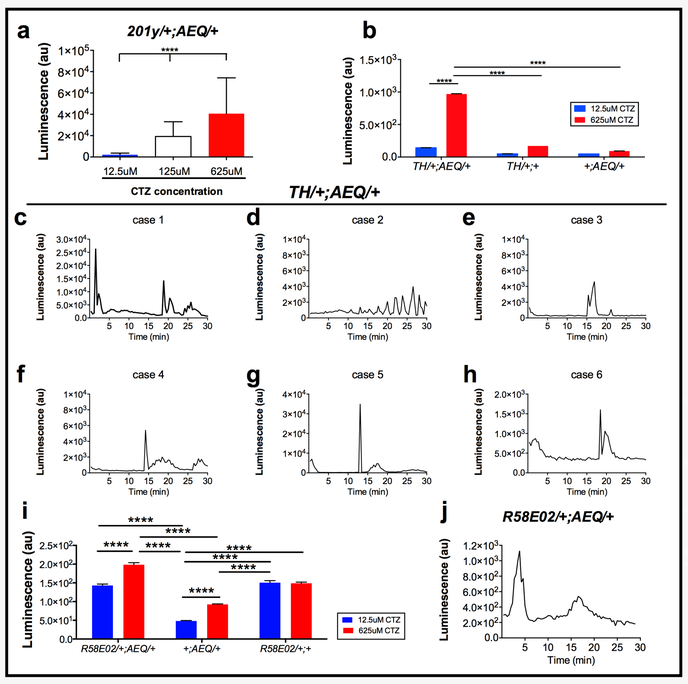

Figure 4. Sensitivity range of the assay from 100s – 6 neurons. **a,b** and **i** show the mean bioluminescence values,**c-h** and **j** are representative individual examples.

Results (II): Detection of neural activity from freely-crawling larvae

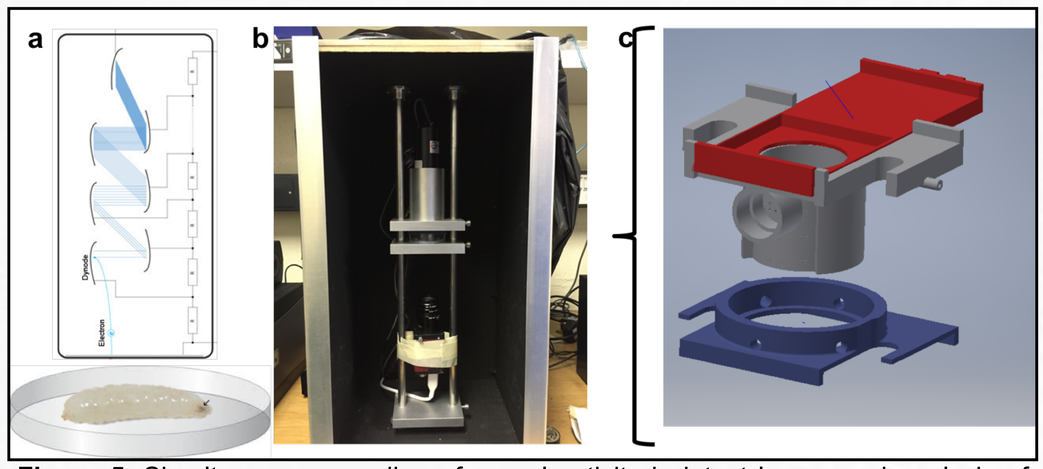

Figure 5. Simultaneous recording of neural activity in intact larvae and analysis of behavior using a photompultiplier in the UV range combined with IR video recording and tracking.

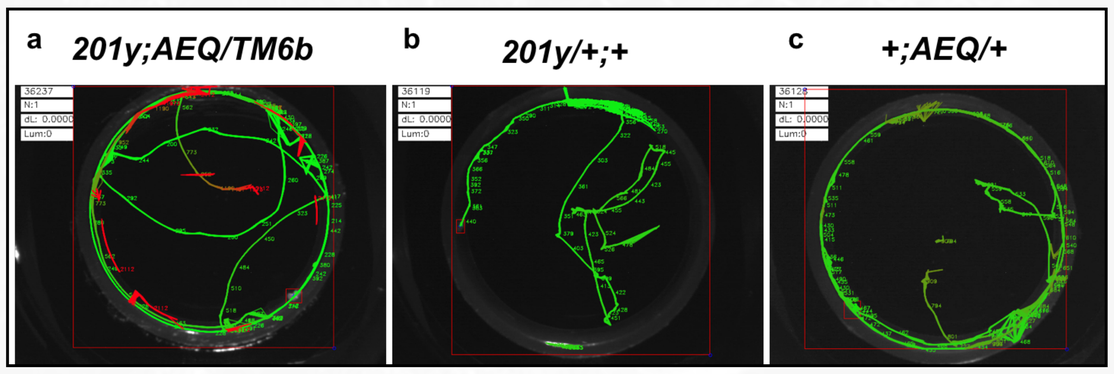

Figure 6. Pairing of neural activity in Drosophila larvae with movement in an arena.

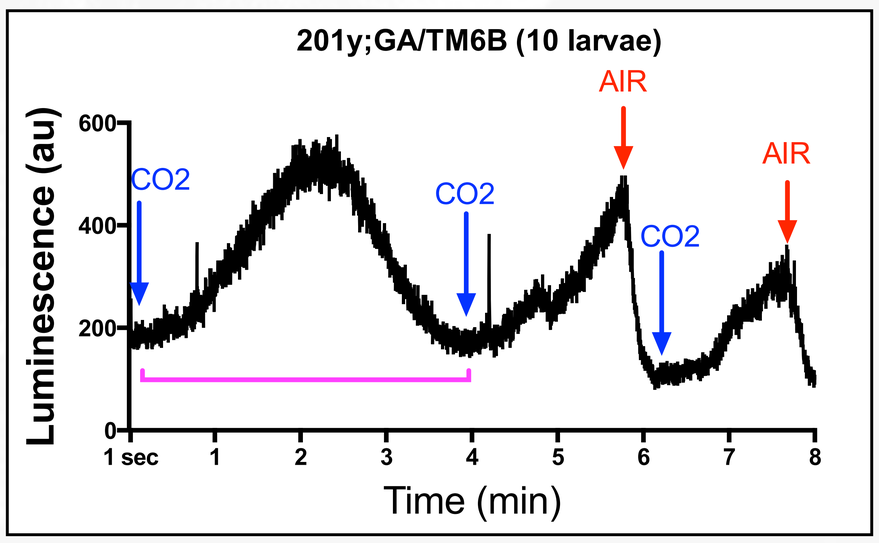

Figure 7. Kenyon cells show increased activity in response to exposure to environmental CO2. Bioluminescence from representative samples shown over time (before and after presenting CO2 to the larva.

Conclusions and Ongoing work:

We developed a method allowing real-time analysis of neuronal activity in intact larvae while they are freely moving. Are aims for the future are. This system works for Kenyon cells and Dopaminergic neuron populations.

Ongoing work is looking at extending the range of stimuli in naïve and trained animals. We are also testing other neuronal populations.

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